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The Effect of Temperature, pH, Sodium Chloride, and Glucose on the Survival of Female *Thelastoma bulhoesi* (Nematoda: Oxyurata)

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ABSTRACT: Female *Thelastoma bulhoesi,* parasites of the hindgut of *Periplaneta americana,* were exposed to a variety of temperatures and solutions of variable pH and sodium chloride and glucose concentrations. *Thelastoma bulhoesi* survived more than 10 days at 27°C, in a neutral pH and 1.0% sodium chloride. Most worms could not survive 7°C for longer than 30 min. Other concentrations of hydrogen ion, sodium chloride, and glucose were less well tolerated but gave unusual bimodal results.

KEY WORDS: *Thelastoma bulhoesi, Periplaneta americana,* bionomics, Nematoda, Oxyuroidea.

Thelastoma bulhoesi is a pinworm (Oyxurata) inhabiting the large intestine of the American cockroach, *Periplaneta americana.* The nematode is common in many laboratory colonies of this host and has been used as an experimental model by many researchers (Lee, 1959, 1960; Guthrie and Tindall, 1968; McCallister and Schmidt, 1983, 1984). It is possible to maintain *Thelastoma bulhoesi* in sterile saline for many hours, making them useful as research and teaching tools. This study presents data that show the effect of temperature and concentrations of sodium chloride (NaCl), glucose, and hydrogen ion on the survival of *T. bulhoesi.* These bionomic data have not been reported previously for this species.

Materials and Methods

Cockroaches, *Periplaneta americana,* were killed in a killing jar using ethyl acetate fumes. The large intestine was removed and teased apart in a 0.75% NaCl solution at a pH of 7. This saline concentration was used when preliminary studies showed best survival of *T. bulhoesi* at this concentration. Female worms were transferred manually, using a bent number 1 insect pin attached to a wooden applicator stick, to autoclaved 65-mm watch glasses containing approximately 2 ml sterile test solutions. These watch glasses were, in turn, maintained in 100% humidity. All experiments were repeated 3 times with between 20 and 30 worms per experiment.

Temperature studies

To determine the effect of temperature on the survival of *T. bulhoesi,* female worms were exposed to temperatures of 0, 5, 15, 25, 35, and 45°C while in 0.75% NaCl at pH 7 and 100% humidity. Worms were removed and examined at ×100 with a compound microscope for motility after 1, 2, 4, 8, 16, 32, 64, 128, and 256 hr. Worms that were not moving spontaneously were agitated with a probe. If they did not respond to the probe, death was assumed. In a separate experiment, female worms were exposed to 5°C for 0.25, 0.5, 1, 2, 4, 8, 16, and 36 hr. At the end of these times, the worms were removed to 26°C incubation chambers, and the length of survival time following cold stress was determined by observing the parasite for motility, as already described, at 1, 2, 4, 8, 16, 32, 63, 128, and 256 hr.

Osmotic and pH studies

Worms were exposed to NaCl at strengths of 0, 0.03, 0.06, 0.12, 0.25, 0.5, 0.75, 1.0, 2.0, and 4.0% at 25°C. These solutions were tested at pH 7 and again at pH 5 to investigate pH effects within the range normally found within the cockroach hindgut (Guthrie and Tindall, 1968). The worms were also exposed to pH 1, 3, 5, 7, 9, and 11 in 0.75% NaCl. Hydrogen ion concentration was adjusted using 1 M HCl or 1 M NaOH. No buffer was added. Glucose was tested at the same weight per volume concentrations as the NaCl, but only at pH 7.

Results

The length of survival of female *T. bulhoesi* at various temperatures is shown in Table 1. Maximum survival occurred at 27°C, where an average of 13% of the organisms survived for more than 10 days. Temperatures of 47 and 7°C were about equally lethal with few worms surviving more than 16 hr. The length of time *Thelastoma bulhoesi* can survive after exposure to 7°C is shown in Table 2. Exposure to 7°C for 30 hr or longer was fatal, but exposure to this low temperature for as little as 30 min affected the survival of the worm, even after it was removed from the stress. Most worms stressed in this manner did not survive past 32 hr postexposure.

The survival of female worms in various concentrations of NaCl is depicted in Tables 3 and 4. Worms were first exposed to NaCl at pH 7 and another group was exposed at pH 5. Survival, as determined by numbers surviving and length of survival, was optimum in 1.0% NaCl at pH 7, where 15% of the worms survived 256 hr. At pH 5 maximum length of survival was in

Table 1. Mean % survival at different temperatures of female *Thelastoma bulhoesi* cultured in 0.75% NaCl, pH 7, 25°C.

Temperature (°C)	Hours								
	1	2	4	8	16	32	64	128	256
0	0	0	0	0	0	0	0	0	0
7	100	100	100	100	27	0	0	0	0
17	100	92	92	92	69	31	7	0	0
27	100	100	95	87	60	60	33	20	13
37	91	82	63	63	64	0	0	0	0
47	100	100	96	39	0	0	0	0	0

0.03% NaCl, where a mean of 10 worms survived for 256 hr. A greater number of worms (40) survived to 128 hr at this pH.

Table 5 shows the results when female worms were exposed to a wider range of pH at 0.75% NaCl. The nematodes survived a large range of pH concentrations. Optimum survival was at pH 7, but about half the worms could survive 64 hr in any pH between 3 and 9.

The mean percentage of survival of female *Thelastoma bulhoesi* in glucose concentrations (wt./vol.) is shown in Table 6. Maximum survival was in 2.0% glucose, and minimum survival was in 0.5% glucose. This creates an interesting bimodal distribution.

Discussion

It is not surprising that parasites of homeothermic animals are limited in the temperature range that they can tolerate. Parasites of poikilotherms might be expected to be more tolerant of temperature extremes because their host is susceptible to environmental temperatures. Much of the research on the effects of temperature on nematodes deals with free-living or plant parasitic forms. Several authors have reviewed the literature on this topic (Lee, 1965; Zuckerman et al., 1971; Nicholas, 1975; Croll, 1976).

Guthrie and Tindall (1968) reported the optimum temperature of *Periplaneta* species to be between 26 and 28°C. It is not surprising to see that the greatest parasite survival was at 27°C. The cockroach host survives temperatures down to 1°C, although with decreased activity. Because *T. bulhoesi* does not survive long at 7°C, holding the host at this temperature for 48 hr might prove to be a method of obtaining worm-free cockroaches for experimental purposes. Nematodes were also affected by temperatures of 47°C, whereas cockroaches can withstand these temperatures depending on the relative humidity. In summary, the temperature tolerance range of female *T. bulhoesi* is within that of their host, generally being more susceptible to extremes than

Table 3. Mean % survival of female *Thelastoma bulhoesi* in concentrations of Nacl incubated at pH 7, 25°C, for varying lengths of time.

NaCl (%)	Hours								
	1	2	4	8	16	32	64	128	256
0.00	100	62	0	0	0	0	0	0	0
0.03	100	88	88	88	88	75	13	0	0
0.06	100	88	77	77	77	77	33	0	0
0.12	100	100	100	89	89	89	38	0	0
0.25	100	100	83	83	83	66	50	0	0
0.50	100	100	100	100	85	66	0	0	0
0.75	100	100	95	87	60	60	35	20	13
1.00	100	100	94	94	94	88	41	29	15
2.00	1	0	0	0	0	0	0	0	0

Table 2. Mean % survival of female *Thelastoma bulhoesi* cultured in 0.75% NaCl, pH 7, 25°C, after exposure to 7°C for different lengths of time.

Exposure time (hr)	Hours								
	1	2	4	8	16	32	64	128	256
0.25	100	90	90	90	65	61	30	15	9
0.50	100	80	80	75	70	60	0	0	0
1.00	100	100	100	85	71	29	0	0	0
2.00	100	100	90	90	80	30	0	0	0
4.00	100	100	90	64	24	19	0	0	0
8.00	100	45	18	18	9	0	0	0	0
16.00	27	27	27	27	18	18	9	0	0
32.00	50	50	42	33	33	0	0	0	0
64.00	8	0	0	0	0	0	0	0	0
128.00	0	0	0	0	0	0	0	0	0

Table 4. Mean % survival of female *Thelastoma bulhoesi* in concentrations of NaCl incubated at pH 5, 25°C, for varying lengths of time.

NaCl (%)	Hours								
	1	2	4	8	16	32	64	128	256
0.00	80	60	20	18	10	0	0	0	0
0.03	100	90	90	90	80	70	40	20	10
0.06	100	80	80	70	70	70	60	40	0
0.12	100	100	83	83	66	66	0	0	0
0.25	100	100	100	100	88	75	50	0	0
0.50	100	100	100	100	100	90	64	18	0
0.75	100	100	100	92	85	62	62	0	0
1.00	100	80	80	70	50	50	30	0	0
2.00	100	73	45	45	27	18	0	0	0
4.00	0	0	0	0	0	0	0	0	0

Table 5. Mean % survival of female *Thelastoma bulhoesi* in variable pH incubated in 0.75% NaCl, 25°C, for varying lengths of time.

Exposure time (hr)	Hours								
	1	2	4	8	16	32	64	128	256
1	38	0	0	0	0	0	0	0	0
3	100	100	91	91	66	64	25	0	0
5	100	100	100	92	85	62	62	0	0
7	100	100	95	87	60	60	33	20	13
9	100	100	92	87	66	55	44	0	0
11	100	100	91	91	64	9	0	0	0

Table 6. Mean % survival of female *Thelastoma bulhoesi* in glucose concentrations maintained at pH 7, 25°C, for varying lengths of time.

Glucose	Hours								
	1	2	4	8	16	32	64	128	256
0.00	80	60	20	18	10	0	0	0	0
0.03	100	64	18	0	0	0	0	0	0
0.06	100	38	38	20	11	0	0	0	0
0.12	100	55	55	32	17	7	0	0	0
0.25	100	35	23	0	0	0	0	0	0
0.50	100	8	0	0	0	0	0	0	0
0.75	100	100	100	100	82	49	0	0	0
1.00	100	100	100	58	58	22	8	0	0
2.00	100	100	100	100	100	100	73	0	0
4.00	100	100	100	100	88	88	55	0	0
8.00	100	100	100	100	51	51	0	0	0
16.00	0	0	0	0	0	0	0	0	0

the cockroach. These ranges also correspond to data reported for other nematodes. The cockroach hindgut varies in osmotic pressure due to drying of the peritrophic membrane during molting. Thus, the nematode must be able to withstand some variation in osmotic pressure in order to parasitize the host continuously through its life cycle. Lee (1966) investigated this phenomenon for *Hammerschmidtiella diesingi,* an oxyurid nematode parasite of the hindgut of *Blatta orientalis.* He showed that the worm could survive the host molt and had some limited abilities of osmoregulation.

The host molting procedure itself takes only 10–20 min (Guthrie and Tindall, 1968), but physiological differences can be noted as early as 2 days before and after (Patton and Flint, 1959; Patton, 1962). While optimum survival of *Thelastoma bulhoesi* occurs at 1% NaCl, the worm can survive for at least 32 hr at any concentration of NaCl tested greater than 0%. It is possible that changes in osmotic pressure may inhibit transtadial transmission of cockroach pinworms. This may account for the results reported by McCallister (1988) that adults are more often, and more heavily, parasitized than nymphs.

The colon of *P. americana* has been determined to have a pH of 7.3 in females and 7.4 for males, using indicator dyes. Using glass electrodes, the pH for both sexes was 7.7 (Guthrie and Tindall, 1968). This probably fluctuates with diet, age, and molting. Because changes in pH can affect solubility of many compounds, it may also have an effect on osmotic pressure. When *T. bulhoesi* is exposed to the same concentrations of NaCl at both pH 5 and 7, the spectrum of survival shifts to lower concentrations of NaCl. Maximum survival concentration at pH 5 is 0.03% NaCl, whereas at pH 7 it is 1.0% NaCl. Optimum survival under normal osmotic pressure appears to occur in solutions of pH 7. This is in keeping with the normal environment in the cockroach hindgut.

Several authors have published data that suggest that the cuticle of nematodes is impervious to sugars (Lee, 1966; Croll and Viglierchi, 1969). This is presumably due to the large size of the molecule and its consequent inability to pass through the cuticle of the nematode. Best survival in glucose solutions in this study was at 2.0%. Death in other concentrations was most likely due to osmotic pressure as nematodes tended to eviscerate or collapse in other concentrations.

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